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An exotic fruit with high nutritional value: *Kadsura coccinea* fruit

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Abstract: This research was to determine nutritional composition, essential and toxic elemental content, and major phenolic acid with antioxidant activity in *Kadsura coccinea* fruit. The results indicated that *Kadsura coccinea* fruit exhibited the high contents of total protein, total fat, ash and essential elements such as calcium (Ca), ferrum (Fe) and phosphorus (P). The levels of four common toxic elements, i.e. cadmium (Cd), mercury (Hg), arsenic (As) and lead (Pb), were lower than legal limits. By high-performance liquid chromatography (HPLC) analysis, gallic acid was identified as major phenolic acid in peel and pulp tissues. Its contents were no significant difference in both tissues. In comparison with two commercial antioxidants, the major phenolic acid extracted from *Kadsura coccinea* exhibited stronger 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity and reducing power. *Kadsura coccinea* fruit is a good source of nutrition and natural antioxidant. It is worthwhile to popularize this exotic fruit around the world.

Keywords: *Kadsura coccinea* fruit, nutritional composition, essential and toxic element, major phenolic acid, antioxidant activity

Introduction

Kadsura coccinea (Lem.) A. C. Smith, a vine plant indigenous to southern China, belongs to the family *Schisandraceae*. Its vine and root are often used as the traditional Chinese medicine for treating gastroenteric disorder and rheumatoid arthritis (Liu and Li, 1995a). *Kadsura coccinea* is well-known as 'black tiger' in China. The fresh fruit of this plant has been consumed by more and more people in some provinces of China such as Guangxi, Guizhou, Yunnan and Guangdong. The exotic fruit is sometimes been processed into juice and wine. Owing to its health benefits, *Kadsura coccinea* fruit deserves attention and needs further exploitation. The previous researches focused largely on the medicinal functions of *Kadsura coccinea*, which proved that some of lignans and triterpenoids presenting in stem or seed extracts were effective as the antitumor, anti-HIV, anti-lipid peroxidative, cytotoxic, and anti-hepatitis agents (Liu and Li, 1995b; Gao *et al*., 2008). In our previous report (Sun *et al*., 2009), it was also found that polyphenol and anthocyanin extracts from *Kadsura coccinea* fruit possessed the good health benefits. Up to now, little information is available on evaluation of nutritional quality, essential and toxic element, and phenolic acid with antioxidant activity in *Kadsura coccinea* fruit, which requires to be investigated prior to further development of this rare fruit.

In biological system, the oxidative stress has been associated with the pathogenesis of many human diseases (Aktan *et al*., 2003; Mariani *et al*., 2005; Karp and Koch, 2006). The application of natural antioxidants in pharmacology can improve current treatments for these diseases. Fruits usually contain phenolic acids, flavonoids and hydrolysable and condensed tannins. These compounds exhibit the strong antioxidant activity (Heim *et al*., 2002; Sroka and Cisowski, 2003) and possess the ability to scavenge both active oxygen species and electrophiles (Robards *et al*., 1999). *Kadsura coccinea* fruit could possibly contain a significant amount of phenolic acids like other plants in *Schisandraceae* family, and therefore it is a good resource of natural antioxidants. The objective of the present study was to evaluate nutritional quality, detect essential and toxic elemental content, and identify major phenolic acid with antioxidant activity of *Kadsura coccinea* fruit. The study is helpful to elucidate health benefit and safety of *Kadsura coccinea* fruit so as to further exploit this exotic fruit and introduce it into more areas.

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Materials and Methods

Plant materials

Fifty mature fruits of *Kadsura coccinea* (Lem.) A. C. Smith with purplish-red pericarp tissues were respectively harvested from ten vines in an orchard of Rongshui, Guangxi in October, 2008. Every fruit was aggregated by about 20~65 small berries. The individual berry without any defects and diseases was selected and picked from the aggregated fruits. Seeds of these berries were discarded while peels and pulps were separated, collected, weighted and then stored at -20°C until further extraction and analysis.

Chemicals and reagents

Commercial standards for HPLC analysis, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The HPLC-grade organic reagents were obtained from Fisher Scientific (Waltham, MA, USA) while ascorbic acid and 2,6-ditertbutyl-4-methylphenol (BHT) were purchased from Aladdin Reagent Inc. (Shanghai, China). Other chemicals and reagents were of analytical grade.

Analysis of nutritional compositions

The edible portion (i.e. pulp tissues) of *Kadsura coccinea* fruits was homogenized and then filtered through muslin. The filtrate was centrifuged at 3500×*g* for 15 min and then the supernatant was collected to analyze the contents of total soluble solids (TSS), titratable acidity (TA) and ascorbic acid. TSS content was determined at 20°C with a hand-refractometer (CANY Co., Shanghai, China). TA content was assayed by titration with 0.1 M NaOH to pH 8.2 and expressed as malic acid content. Ascorbic acid content was evaluated with a reagent kits from Jiancheng Bioengineering Institute (Nanjing, China) on a fresh weight (FW) basis. Total protein content in the edible portion was estimated by Kjeldahl method while total fat content was assayed by Soxhlet method described by AOAC (1990). The ash content was determined by the ignition method using the crucible and muffle furnace.

Determination of essential and toxic elemental contents

Determination of elemental contents was performed according to the official analytical methods (AOAC, 2000). Contents of calcium (Ca) and ferrum (Fe) were measured using AA-6800 atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan) while phosphorus (P) content was estimated in a T_6 spectrophotometer (Purkinje general Instrument Co., Beijing, China). Flame atomic-absorption spectrometry (PerkinElmer Analyst 800, PerkinElmer Inc, Norwalk, CT, USA) was used to quantify the contaminants cadmium (Cd) and lead (Pb). The procedure was based on sample incineration (5 g) and dissolution of tissues in HNO_3. Concentrations were calculated from linear calibration plots obtained by measurement of standard solutions absorbance: $Cd(NO_3)_2$ and $Pb(NO_3)_2$ (1 g/L dissolved in 0.5 M HNO_3). Mercury (Hg) and arsenic (As) were measured on an AFS-230E atomic fluorescence spectrometer (Kechuang Haiguang Instrument Co., Beijing, China) using the mercury and arsenic vapour generation technique.

Extraction of major phenolic acid

This step was performed according to a modified method of Zhang *et al*. (2000). The crushed fresh peel or pulp tissues (20 g) were successively extracted twice for 30 min in a shaking incubator (ZHWY-200B, Zhicheng Analytical Co., Shanghai, China) at 20ºC, using 100 mL of methanol/acetone/water (3.5/3.5/3, v/v/v) containing 1% (v/v) formic acid. These extracts were combined and then filtered through two layers of cheesecloth. The collected filtrate was centrifuged for 15 min at 7000×*g*. The supernatant was collected and the total phenolic (TP) content in the edible portion was determined using Folin–Ciocalteau method (Singleton and Rossi, 1965). TP content was standardized against gallic acid and expressed as microgram per milliliter of gallic acid equivalents (GAE). In addition, the supernatant was further filtered through a 0.45 μm syringe filter (Millex-HV, Millipore Co., Billerica, MA, USA) for HPLC analysis of major phenolic acid.

HPLC analysis

The HPLC analysis on extracts from *Kadsura coccinea* peel or pulp tissues were performed according to the modified method of Zhang *et al*. (2000). The Ultimate 3000 HPLC system (Dionex, Sunnyvale, CA, USA) equipped with a Venus C_{18} column (250 × 4.6 mm, 5 μm particle size; Dalian Create Science and Technology, Dalian, China) and a UV detector (Dionex) was used for the identification of individual compound while chromeleon chromatography management software (Dionex) was used for data recording and processing. The mobile phase was (A) acidic water (2% acetic acid) and (B) acetonitrile-methanol (10/15, v/v). The gradient elution was as follows: 0 min, 90% A; 10 min, 80% A; 15 min, 70% A; 25 min, 60% A; 30 min, 50% A; 40 min, 50% A; 45 min, 90% A (the initial condition)

Table 1. Contents of nutritional compositions, essential and toxic elements in the edible portion of *Kadsura coccinea* fruit [a]

Nutritional compositions	Contents	Elements	Contents
Total soluble solids (°Brix)	7.2 ± 0.3	Ca (mg/g)	3.8 ± 0.7
Titratable acidity (g/100g)	0.16 ± 0.02	Fe (μg/g)	29 ± 2.3
Ascorbic acid (mg/100g)	3.3 ± 0.4	P (mg/g)	0.57 ± 0.08
Total protein (g/100g)	3.1 ± 0.7	Cd (μg/g)	< MDC
Total fat (g/100g)	1.9 ± 0.1	Pb (μg/g)	0.05
Ash (g/100g)	7.4 ± 0.5	Hg (μg/g)	< MDC
Total phenolic (μg/ml GAE)	81.1 ± 1.6	As (μg/g)	< MDC

MDC, minimum detectable concentration (0.01 μg/g);
[a] Each data point represents the mean ± SE.

and then held for 5 min before next injection. The flow rate and injection volume were 1 mL/min and 10 μL, respectively. The monitoring wavelength was 280 nm. Identification of the individual compound was achieved by comparison with retention times (Rt) of standards, UV spectra and calculation of UV absorbance ratios after co-injection of samples and standards. Furthermore, the major phenolic acid was collected and quantified by a comparison with a multipoint calibration curve obtained from the corresponding standard. Serial dilution of the standard solutions at 0.05–2 mg/mL using ethanol was performed.

DPPH radical-scavenging activity of major phenolic acid

The DPPH-scavenging activity was estimated according to the modified method of Sun *et al*. (2007). Aliquots (0.5 mL) at 0 (control), 25, 50, 100, 250, 500 and 1000 μg/mLof major phenolic acid dissolved in ethanol were added into 2.5 mL of 0.2 mM DPPH solution in ethanol. The absorbance at 517 nm of samples was measured after 30 min of incubation at 26°C in the dark using a UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). DPPH-scavenging activity (%) = $1 - [(A - B) / A_0] \times 100\%$, where A = absorbance of sample, B = absorbance of 0.5 mL of major phenolic acid + 2.5 mL of ethanol, and A_0 = absorbance of control. The calculation of 50% inhibition concentration (IC_{50}) to scavenge 50% of radical was obtained according to the method of Senevirathne *et al*. (2006). Two commercial antioxidants (ascorbic acid and BHT) were used as positive controls.

Ferric reducing/antioxidant power (FRAP) of major phenolic acid

FRAP assay was conducted according to the modified method of Benzie and Strain (1996). The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10/1/1 ratio prior to use and then heated to 37°C in a water bath. A total of 3.0 mL of FRAP reagent was added to a test tube and a blank reading was then taken at 593 nm using the spectrophotometer. A total of 100 μL of 0−1000 μg/mL major phenolic acid dissolved in ethanol and 300 μL of distilled water were added to the cuvette. After the addition of the FRAP reagent, a second reading at 593 nm was performed after 40 min of incubation at 37°C in a water bath. The changes in absorbance after 40 min from the initial blank reading were compared with the standard curve. Standards of known Fe (II) concentrations were used at 200−1000 μM and then a standard curve was prepared by plotting the FRAP value of each standard versus its concentration. The result was expressed as the concentration of antioxidant exhibiting a ferric reducing ability per gram of sample, μM/g.

Data analysis

All experiments were performed in triplicate (n=3). The results represented mean ± standard error (SE) of three replicated determinations. An ANOVA test (SPSS 13.0 statistical software, SPSS Inc., Chicago, USA) was used to compare the mean value of each treatment. Significant differences between the means were determined by using the LSD test ($P<0.05$).

Results and Discussion

Nutritional compositions

In the present study, total soluble solids, titratable acidity and total protein contents of *Kadsura coccinea* fruit from Guangxi were 7.2 ± 0.3 °Brix, 0.16 ± 0.02 g/100g and 3.1 ± 0.7 g/100g, respectively (Table 1). The contents of three nutritional compositions were similar to the previous report on this fruit planted in Guizhou (Tao and Ou, 2003). Furthermore, by comparison, under the same conditions, total protein content in *Kadsura coccinea* fruit was significantly higher ($P<0.05$) than that in some common fruits with similar taste such as grape (cv. Hongmeigui, 0.4 ± 0.05 g/100g), apple (cv. Hongfushi, 0.7 ± 0.2 g/100g) and banana (cv. Huangdijiao, 1.2 ± 0.1 g/100g). Total fat content in *Kadsura coccinea* fruit (1.9 ± 0.1 g/100g) was also significantly higher

($P<0.05$) than that in three common fruits (grape 0.3 ± 0.03 g/100g, apple 0.4 ± 0.1 g/100g and banana 0.6 ± 0.2 g/100g). Above results indicated that *Kadsura coccinea* fruit exhibited high nutritional values in terms of total protein and total fat. In addition, ash content of *Kadsura coccinea* fruit was relatively higher (7.4 ± 0.5 g/100g). Ascorbic acid and total phenolic contents of this fruit were determined as 3.3 ± 0.4 mg/100g and 81.1 ± 1.6 µg/mL GAE. Ascorbic acid and phenolic compounds were closely relative to antioxidant activities of *Kadsura coccinea* fruit.

Essential and toxic elemental contents

Trace elemental levels in *Kadsura coccinea* fruit are listed in Table 1 on a wet weight basis. The contents of Ca (3.8 ± 0.7 mg/g), Fe (29 ± 2.3 µg/g) and P (0.57 ± 0.08 mg/g) were very high in this fruit. This result was similar to the report by Huang *et al*. (2006) who found that Ca, Fe, Mn, Cu, Zn and Mg contents in *Kadsura coccinea* vine were obviously higher than those in other sixteen vegetables (e.g. spinach, radish, taro, cabbage, lettuce, eggplant, garlic, shallot, leek, celery, etc.). The nutritional elements such as Ca, Fe and P are considered essential for the maintenance of normal metabolic and physiological functions of human body. These essential elements play key roles in bone metabolism or skeletal integrity, muscular activity, reproduction and development (Matkovic and Heaney, 1992; Ilich and Kerstetter, 2000; Speich *et al*., 2001).

Four major toxic elements (Cd, Hg, As and Pb) were further detected in this study. In human body, the accumulation of Cd may induce kidney dysfunction, skeletal damage and reproductive deficiencies. Hg is toxic to the developing fetus and considered a possible carcinogen. As is also a carcinogen, causing lung, liver, skin, bladder cancer. Pb can induce reduced cognitive development and intellectual performance in children and increase blood pressure and cardiovascular disease in adults (Tuzen, 2009). In *Kadsura coccinea* fruit, the contents of toxic elements Cd, Hg and As were found under the minimum detectable concentration (MDC, 0.01 µg/g). The maximum Cd, Hg and As levels permitted for fruits are 0.03 mg/kg, 0.01 mg/kg and 0.5 mg/kg according to National Standard of the People's Republic of China (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, 2001). Above three toxic element levels in *Kadsura coccinea* fruit samples were found to be lower than legal limits. In addition, Pb contents in this fruit was determined as 0.05 ± 0.006 µg/g which was also lower than the maximum Pb level (0.2 mg/kg) legally permitted for fruits (National Standard of the People's Republic of China).

Determination of major phenolic acid

Through HPLC analysis, the Rt of gallic acid standard was around 5.1 min (Figure 1a). In the HPLC profiles of *Kadsura coccinea* peel and pulp tissues, only a single peak appeared at 5.13 min for peel extract (Figure 1b) and 5.08 min for pulp extract (Figure 1c), respectively. The single peak in both tissues possessed the same Rt and UV spectra with gallic acid standard, indicating that gallic acid (an important base unit of hydrolysable tannins) was the major phenolic acid in peel and pulp tissues of *Kadsura coccinea* fruit. This compound was also found in other plants of the family *Schisandraceae*. By using the calibration with standard curve ($Y = 514.35X - 19.569$ and $r^2>0.99$; X, the concentration of gallic acid standard, and Y, peak area), the gallic acid contents in peel and pulp tissues were calculated as 1129.95 ± 4.79 mg/100g FW and 1128.76 ± 2.38 mg/100g FW. Both had no significant difference. Except for gallic acid, there are no other obvious peaks of phenolic acid or derivatives to be found in Figure 1. It could be due to the present in the significant amount of gallic acid, causing other peaks unable to appear distinctly on HPLC profiles.

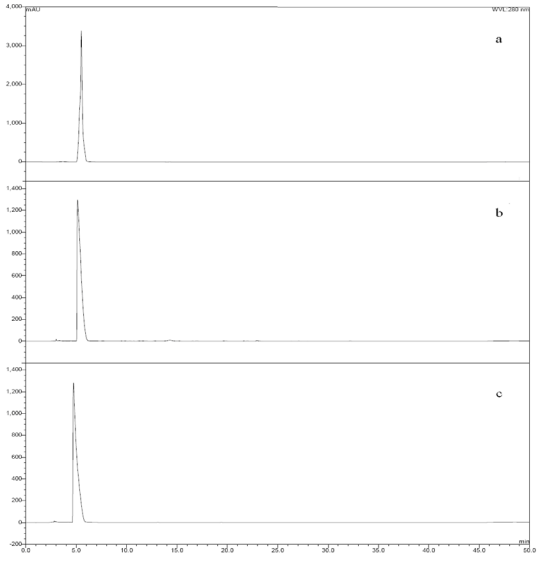

Figure 1. HPLC chromatograms of gallic acid standard (a) and phenolic acid extracts from peel tissues (b) and pulp tissues (c) of *Kadsura coccinea* fruit

Many researches have reported that phenolic acid, flavanols and their derivatives exhibit strong free radical scavenging activities and antioxidant capabilities *in vivo* and *in vitro*, suggesting that they play an important role in improving immunity, inhibiting mutagenesis or preventing against cancer, inflammatory and cardiovascular diseases (Kondo *et al*., 1999; Lin *et al*., 1999; Yilmaz and Toledo, 2004; Lu *et al*., 2006). Through the present study, *Kadsura*

coccinea fruit contained considerable phenolic acids (especially gallic acid) and therefore had strong antioxidant potential. This exotic fruit would probably exhibit biologic effects in terms of health promotion.

DPPH radical-scavenging activity of major phenolic acid

Free radical-scavenging activity is one of the known mechanisms by which antioxidants inhibit lipid peroxidation (Duan *et al*., 2007). The DPPH radical-scavenging activity has been extensively used for screening antioxidants from fruits. Figure 2 shows the DPPH radical-scavenging activity of major phenolic acid in *Kadsura coccinea* fruit. At low concentrations such as 25 and 50 μg/mL, the DPPH radical-scavenging activity of major phenolic acid was significantly ($P<0.05$) higher than that of two common commercial antioxidants (ascorbic acid and BHT). In the concentration range from 100 to 1000 μg/mL, major phenolic acid exhibited a similar DPPH-scavenging activity to ascorbic acid, but it possessed significantly higher ($P<0.05$) scavenging activity than BHT. In addition, in this study, DPPH-scavenging activity was linearly correlated with the concentrations of major phenolic acid, ascorbic acid and BHT in the range from 0 to 50, 0 to 100 and 0 to 1000 μg/mL. The corresponding correlation coefficients were 0.908 for major phenolic acid ($Y = 1.762X + 8.3467$), 0.9858 for ascorbic acid ($Y = 0.9507X + 3.93$) and 0.9744 for BHT ($Y = 0.0462X + 4.9108$). By the calculation of 50% inhibition concentration, it was further confirmed that major phenolic acid (IC_{50} 23.6398 ± 0.1273 μg/mL) had the strongest DPPH radical-scavenging activity, followed by ascorbic acid (IC_{50} 48.4590 ± 0.7446 μg/mL) and BHT (IC_{50} 975.9567 ± 33.9536 μg/mL).

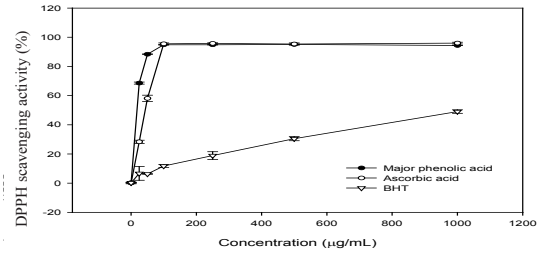

Figure 2. Comparison of DPPH scavenging activity (%) of major phenolic acid and commercial antioxidants

FRAP assay of major phenolic acid

FRAP assay is another important method to evaluate the total antioxidant capacity of biological samples, depending upon the reduction of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine with an intensive blue color by a reductant at low pH (Benzie and Strain, 1996). As shown in Figure 3, major phenolic acid from *Kadsura coccinea* fruit had higher FRAP values than both commercial antioxidants in the concentration range of 25−1000 μg/mL, indicating that major phenolic acid exhibited the strongest ability to reduce Fe (III) to Fe (II). At high concentrations (500 and 1000 μg/mL), the FRAP value of major phenolic acid was similar to that of ascorbic acid, but significantly ($P<0.05$) higher than that of BHT. Furthermore, the FRAP value was positively correlated with the concentrations of major phenolic acid, ascorbic acid and BHT in the ranges from 0 to 250, 0 to 500 and 0 to 1000 μg/mL, and the corresponding correlation coefficients were determined to be 0.9297 for major phenolic acid ($Y = 2.9409X + 297.1$), 0.9834 for ascorbic acid ($Y = 1.4938X + 254.42$) and 0.9999 for BHT ($Y = 0.4405X + 217.48$).

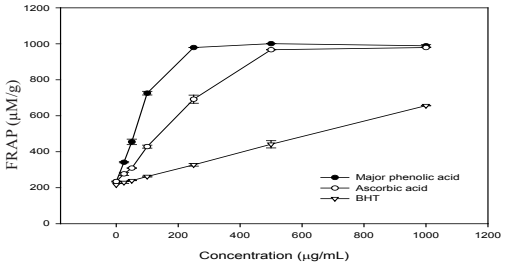

Figure 3. Comparison of reducing power (FRAP values) of major phenolic acid and commercial antioxidants

Conclusions

In this study, *Kadsura coccinea* fruit demonstrated higher contents of total protein, total fat, ash and microelement (Ca, Fe and P) in the edible portion. Four common toxic element (Cd, Hg, As and Pb) levels in this fruit were lower than legal limits. In addition, *Kadsura coccinea* fruit contained a large number of phenolic acids, especially gallic acid, in peel or pulp tissues. Antioxidant activity evaluations showed that major phenolic acid in *Kadsura coccinea* fruit possessed stronger DPPH radical-scavenging activity and reducing power in comparison with two common commercial antioxidants. Because of its high nutritional value and strong antioxidant activities, *Kadsura coccinea* fruit possesses potential benefits for human health.

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on *Kadsura coccinea* plant.

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